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# ANTIGENIC AND IMMUNOGENIC PROPERTIES OF NUCLEOPROTEINS OF DYSENTERIC BACTERIA

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[Tables referred to herein are appended.]

In their research on the bacteria of the entero-typhoidal group up to now, the efforts of microbiologists and biochemists have been centered exclusively on repeated analyses of bacterial antigens. As for the nucleoproteins, which often constitute as much as 80 percent of the dry weight of these bacteria, they were ignored by the investigators.

Research on the antigenic and immunogenic characteristics of nucleoproteins, however, is vital in the clarification of the role of each component of the bacterial cell in immunology.

Our investigations were initially concerned with the nucleoprotein of Flexner's Bacillus (Shigella paradysenteriae).

The nucleoprotein was obtained from the bacterial cell which was previously treated with trichloroacetic acid to isolate the whole antigen. The nucleoprotein, obtained by this method, proved to be slightly toxic in animals (a 3-milligram dose was perfectly tolerant for mice). The antigenic properties of nucleoprotein were clearly manifested. Precipitins and also agglutinins of dysenteric bacteria of a low titer appeared in the sera of the immunized animals. However, a detailed study of the precipitative properties of these sera produced very unexpected results. These sera produced positive precipitation reaction when tested with nucleoproteins and the whole antigen. The titer of the precipitins in comparison to the whole antigen was often significantly higher than in the case of the nucleoprotein. Table 1 gives data indicating the properties of the nucleoproteins that were obtained by various methods.



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It might be propounded that this precipitation reaction occurs due to the presence of similar albuminous components in the nucleoprotein and in the albumin content of the antigens. However, these test sera produced positive precipitation reactions not only with the antigens but also with polysaccharose (hapten) from which the albumen had been removed. In addition, it was possible to separate the antibody into antigens and nucleoproteins by the adsorption method.

It is evident that these antisera contained two antibodies: one with respect to the antigens, the other, to the albumen.

The above fact merely proved that the nucleoprotein preparations with which we worked also contained antigens.

We undertook the task of obtaining a nucleoprotein as free from the antigens as possible. Tests were made with nucleoproteins that were obtained from a bacterial growth which was washed three, five, and eight times with trichloroacetic acid to eliminate the antigens as much as possible.

However, the results of this test were identical to the previous test as may be seen from the data in Table 2.

According to Table 2, the antisera, obtained from rabbits immunized with the nucleoproteins which were isolated after repeated washing with trich[illegible] acid, contained precipitins with relation to the albumen, and to the whole antigen. Here also the titer of the antigens was considerably higher than the nucleoprotein.

On the strength of these results, we decided to fractionate the nucleoprotein since it was quite evident that the nucleoprotein isolated directly from the bacterial growth by means of a weak alkaline solution was not an inseparable component. The fractionation was performed by A. N. Belozerskiy's method (1). As a result of this fractionation we obtained a series of products corresponding to the nuclear and cytoplasmatic elements of the bacterial cell according to their chemical properties. Most astonishing fact was that this admixture of basic elements was found in all the fractionated products and it appeared especially clearly in all of those products which corresponded to the nuclear elements of the cell. Immunization of rabbits with nucleoproteins, i.e., with nucleoproteins containing albumen and thymonucleic acid, produced antisera that precipitated a given nucleoprotein in titers of 1:8,000 and the antigens in titers of 1:32,000 - 1:128,000.

Experiments made with nucleoproteins isolated from strains of Flexner's Bacillus and from other typhoidal bacilli gave completely analogous results proving that a common characteristic exists in these phenomena.

The fact that both the repeatedly washed bacterial substance obtained from S-form microbes and the complicated chemical treatment of the nucleoprotein in its fractionation process completely failed in isolating the nucleoprotein from the basic elements suggests that these basic elements enter into an indissoluble organic union with the bacterial nucleoprotein.

We turned to the investigation of the nucleoprotein of R-form dysenteric bacteria. It was established that in the process of immunization, the nucleoprotein of the R-form bacilli produced precipitins only in titers of 1:4,000 - 1:8,000. The latter fully conforms with our data on the antigenic structure of R-form bacilli.

Our subsequent work involved the investigation of the immunogenic properties of the nucleoprotein. The results proved that the basic antigen responsible for the immunization effect is the bacterial antigen. As for the nucleoprotein, its immunization effect is either negligible or completely lacking. Only when immunization is made with large doses is the nucleo-

protein capable of producing some immunization effect. It is quite probable that this minor immunization effect is due to the remnants of the whole antigen.

The great amount of experimental material which has been at our disposal in the course of several years work has convinced us that the S-form nucleoproteins contain a negligibly small amount of the specific antigenic property. This basic element may be either a specific polysaccharide connected with the nucleoprotein molecule or a whole antigen.

We attempted to solve this problem by treating the nucleoprotein 0.1 N by boiling in acetic acid, to decompose the whole antigen if present. The study of the antigenic properties treated by this method showed that the preparation lost its basic element. The resulting antisera produced positive reactions only against nucleoproteins, and negative reactions against the whole antigen and polysaccharide. These tests prove that the basic element of nucleoprotein is most likely the whole antigen which, by the above-mentioned treatment, turned into hapten, the polysaccharide deprived of all antigenic properties.

The assumption that the whole antigen is the basic element united with the nucleoprotein is strengthened by the data of Boivin (3) who demonstrated that the whole antigen can be divided in its entirety only after the ingestion of the bacterial growth by proteolytic ferments.

How should we treat the material we have obtained through experiments? Above all, we most decisively disagree with the proposition that the corresponding antigenic properties of nucleoproteins which we have studied are influenced by their compounding with the whole antigen. Against such a conception, there exists the fact that the whole antigen may be isolated in its entirety from the nucleoprotein only by the action of proteolytic ferments which completely destroy the nucleoprotein molecule. On the other hand, whatever the method used (fractionation, multiple washing with trichloracetic acid, etc.,) as long as an intact nucleoprotein molecule exists it will always produce reactions simulating the presence of a whole antigen.

Our experimental data was confirmed by the work of A. G. Kravchenko and A. I. Larkin (4) which has just been published.

On the basis of all the experimental data we have at our disposal, we come to the conclusion that the main components of the whole antigen localize themselves on the exterior of the cell and can be easily extracted. Another insignificant component of the whole antigen is organically united with elements of the protoplasm of the bacterial cell and, in particular, with the nuclear elements. This component of the whole antigen, organically united with the bacterial nucleoproteins, has a antigenic significance which has been pointed out earlier by Peshkov and Belozerskiy.

The study of the chemical composition of the protoplasm of the R-form of Flexner's Bacillus and the study of the antigenic and immunogenic properties of the R-form nucleoprotein proved very important.

No quantitative or qualitative change was noted in the composition of the basic substances of the protoplasm in the R-form of the bacteria in comparison with the S-form. The sole change observed in the R-form was the complete disappearance of the whole antigen: The transition in the R-form is attended not only by the loss of the superficially distributed whole antigen, but also by the loss of that component which is organically united with the nuclear elements of the bacterial cell.

The extremely close relation between the whole antigen and the nucleoproteids and, in particular, between the whole antigen and the atomic nucleoproteids is extremely evident and has great biological significance.

This, evidently, is the source of the function of the superficially localized whole antigen. The transition into the R-form is accompanied by the loss of this reproductive power.

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[Appended tables follow.]

Table 1. ANTIGENIC PROPERTIES OF NUCLEOPROTEIN

| Type of antigen | Series of immunization | Amount of antigen injected (in milligrams) | Average titer of agglutinins | Average titer of precipitins regarding Nucleoprotein | Whole antigen |
|---|---|---|---|---|---|
| Nucleoprotein (according to Belozerskiy (1)) | 4 | 5 | 1:6400 | 1:8000 | 1:12,000 - 1:64,000 |
| Same as above | 3 | 1.5 | 1:2400 | 1:8000 | 1:12,000 - 1:64,000 |
| Nucleoprotein (according to Konnikov (2)) | 4 | 2.5 | 1:6400 | 1:8000 | 1:16,000 |

Table 2. ANTIGENIC PROPERTIES OF NUCLEOPROTEINS OBTAINED BY REPEATED WASHINGS OF FLEXNER'S BACILLUS

| No of rabbits | Number of washings by trichloroacetic acid | Titer producing precipitation reactions For the whole antigen | For the nucleoprotein |
|---|---|---|---|
| 15 | 1 | 1:32,000 | 1:4,000 |
| 40 | 1 | 1:64,000 | 1:8,000 |
| 9 | 1 | 1:32,000 | 1:8,000 |
| 35 | 3 | 1:64,000 | 1:16,000 |
| 40 | 5 | 1:128,000 | 1:8,000 |
| 38 | 5 | 1:64,000 | 1:8,000 |
| 50 | 5 | 1:64,000 | 1:8,000 |
| 56 | 5 | 1:8,000 | 1:2,000 |
| 79 | 5 | 1:64,000 | 1:4,000 |
| 80 | 8 | 1:128,000 | 1:16,000 |
| 81 | 8 | 1:128,000 | 1:16,000 |

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